

CLAIMS

We claim:

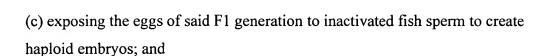
- 1. A method of identifying a gene involved in cell proliferation comprising the steps of:
 - (a) exposing a fish to a mutagen;
 - (b) mating said fish with a wild-type fish to produce an F1 generation;
 - (c) exposing the eggs of said F1 generation to inactivated fish sperm to create haploid embryos; and
 - (d) screening said haploid embryos for cell proliferation defects wherein an embryo with cell proliferation defects harbors a mutant gene involved in cell proliferation.
- 2. The method of claim 1, further comprising a steps of:
 - (e) mating the F1 generation with wild-type male fish to produce an F2 generation;
 - (f) raising said F2 generation to adulthood;
 - (g) mating a female member of the F2 generation with a male member of the F2 generation to produce F3 embryos;
 - (h) screening the F3 diploid embryos for cell proliferation defects wherein an embryo with cell proliferation defects harbors a mutant gene involved in cell proliferation.
- 3. The method of claim 1, wherein the fish in the step (a) is à male fish.
- 4. The method of claim 1 wherein the fish is a zebrafish.
- 5. The method of claim 1, wherein the mutagen is an alkylating agent.

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- 6. The method of claim 1 selected from a group consisting of ENU and MNU.
- The method of claim 1 or 2 further comprising a step of positional cloning of a nucleic 7. acid sequence of the mutant gene.
- 8. The method of claim 1 or 2, wherein the screening is performed using an antibody against a cell cycle component.
- 9. The method of claim 8, wherein the antibody is specific for a protein selected from the croup consisting of phospho-histone H3, phosphorylated MAP kinase, phosphorylated MEK-1, BM28, cyclin E, p53, Rb and PCNA
- 10. The method of claim 1 or 2, wherein the screening is performed using nucleic acids recognizing cell cycle components.
- 11. The method of claim 9, wherein the nucleic acid is PCNA or cyclin b-1.
- The method of claim 1 or 2, wherein the creening is performed using flow cytometry. 12.
- The method of claim 1 or 2, wherein the scheening is performed using apoptosis markers. 13.
- 14. The method of claim 13, wherein the apoptosis marker is selected from the group consisting of Annexin V, TUNEL Stain, 7-amino-actinomycin D and Caspase substrates.
- 15. The method of claim 1 or 2, wherein the screening is preformed using BrdU staining.
- 16. The method of claim 1 or 2, wherein the screening is performed using an irradiation analysis.
- The method of claim 1 or 2, further comprising a step of positional cloning of the gene 17. involved in cell proliferation.
- 18. A method of identifying a gene involved in carcinogenesis comprising the steps of:
 - (a) exposing a fish to a mutagen;
 - (b) mating said fish with a wild-type fish to produce an F1 generation;



- (d) screening said haploid embryos for cell proliferation defects wherein an embryo with cell proliferation defects harbors a mutant gene involved in cell proliferation;
- (e) mating an F1 generation female of step (c) harboring a mutant gene involved in cell proliferation as determined in step (d) with a wild-type fish to produce an F2 generation;
- (f) exposing a wild-type fish and a member of the F2 generation to a carcinogen; and
- (g) comparing the tumor formation in the wild-type and the member of the F2 generation fish wherein an accelerated tumor formation in the F2 generation fish indicates a gene involved in carcinogenesis.
- 19. The method of claim 18, wherein the fish is a zebrafish.
- 20. The method of claim 18, further comprising a step of positional cloning of the gene involved in carcinogenesis.
- 21. The method of claim 18, wherein the screening is performed using an antibody against a cell cycle component.
- 22. The method of claim 21, wherein the antibody is specific for a protein selected from the croup consisting of phospho-histone H3, phosphorylated MAP kinase, phosphorylated MEK-1, BM28, cyclin E, p53, Rb and PCNA.
- 23. The method of claim 18, wherein the screening is performed using nucleic acids recognizing cell cycle components.
- 24. The method of claim 23, wherein the nucleic acid is PCNA or cyclin b-1.
- 25. The method of claim 18, wherein the screening is performed using flow cytometry.

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- 26. The method of claim 18, wherein the screening is performed using apoptosis markers.
- 27. The method of claim 26, wherein the apoptosis marker is selected from the group consisting of Annexin V, TUNEL Stain, 7-amino-actinomycin D and Caspase substrates.
- 28. The method of claim 18, wherein the screening is preformed using BrdU staining.
- 29. The method of claim 18, wherein the screening is performed using an irradiation analysis.

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